was collected every 3 days and replaced with new medium containing freshly added FGFs. The explants were removed after 15 days, frozen in liquid nitrogen and stored (along with their culture medium) at -20 °C. The concentration of glycosaminoglycans (GAGs), a measure of cartilage breakdown, was determined in the culture medium using the dimethylmethylene blue (DMMB) assay. Histological changes in the cartilage were observed by staining frozen, transverse sections (7 μm) of the explants with toluidine blue.

Results: Impact load markedly increased the release of GAGs into the medium compared to unloaded control explants after 3 (P=0.003), 6 (P=0.015), 9 (P=0.017) and 12 (P=0.046) days in culture. Addition of either FGF2 or FGF18 had no significant effect on the release of GAGs into the medium at any concentration or time point. In histological sections of unloaded control explants, the normally flattened chondrocytes in the surface zone became more rounded when FGF2 or FGF18 (100 ng/ml) was added to the medium for 15 days and some cell proliferation was observed. Impact loaded explants showed characteristic damage and fissuring in the articular surface. In the presence of FGF2 and FGF18 (100 ng/ml), marked chondrocyte proliferation, with clusters of new rounded cells, was observed in the surface zone and around the fissures. FGF2 treatment was found to show more distinct changes than FGF18.

Conclusions: In this study FGF2 and FGF18 treatment apparently was unsuccessful in preventing GAG release, and therefore cartilage breakdown, following an impact at the concentrations used. However this is the first time, as far as we are aware, that FGF18 has been shown to promote cell proliferation following a traumatic load. A window of opportunity may exist, soon after joint injury has occurred, during which the addition of this growth factor may promote a repair response and thus slow down the progression of cartilage degradation.

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OXIDIZED LOW DENSITY LIPOPROTEIN (OX-LDL) INDUCES HYPERTROPHIC CHONDROCYTE-LIKE PHENOTYPES IN CULTURED BOVINE ARTICULAR CHONDROCYTES

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Purpose: It has been suggested in-vivo and in-vitro studies that ox-LDL can be a factor related to degeneration of articular cartilage. Meanwhile, it has been reported that chondrocytes in the degenerative cartilage with fibrillation are activated to show hypertrophic chondrocyte-like phenotypes, including up-regulation of type X collagen expression, down-regulation of type II collagen expression, morphologic changes and other distinct gene expressions. The purpose of this study is to investigate effects of ox-LDL on expression of type X and II collagen, and hypoxia induced factor (HIF) family in cultured bovine articular chondrocytes (BACs).

Methods: BACs were isolated and cultured in monolayer reaching 100% confluence at 37°C in a humidified and hypoxic atmosphere (5% O_2 and CO_2). First, we investigated effects of ox-LDL addition on number of nodules formed by cultured BACs. Dose responses of type X and II collagen and HIF-2alpha mRNA expression 24 hrs after ox-LDL stimulation were investigated using quantitative Real-time RT-PCR (reverse delta-delta Ct method). We also investigated effects of ox-LDL on these gene expressions under existence of N-acethyl cysteine (NAC, anti-oxidant) and LY294002 (PI3K specific inhibitor). Protein expressions of type X collagen were investigated by immunofluorescent cell staining and western blot analysis. And we used siRNA for lectin-like ox-LDL receptor-1 (LOX-1), a receptor for ox-LDL expressed on chondrocytes, to demonstrate whether LOX-1 mediates these effects of ox-LDL.

Results: Number of nodules formed by BACs on dishes after ox-LDL treatment was more than that on control dishes. Expression of type X collagen was upregulated both in mRNA and protein levels by addition of 10 to 50 μg/ml ox-LDL. Expression of type II collagen was suppressed by ox-LDL addition in a dose-dependent manner. Immunofluorescent cell staining showed an increase in type X collagen production after ox-LDL stimulation (Figure 1). And expression of HIF-2alpha was upregulated in mRNA by ox-LDL. These effects of ox-LDL were suppressed by pretreated by NAC and LY294002. Upregulation of type X collagen expression by ox-LDL stimulation was attenuated in LOX-1-knockdown cells.

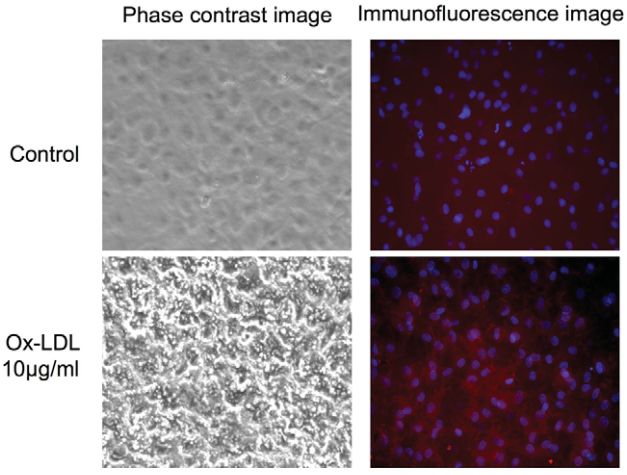

Figure 1. Immunofluorescent cell staining for type X collagen. Ox-LDL of 10 μg/ml increased type X collagen production which is shown by red color (10 days after ox-LDL stimulation). Original magnification is ×400.

Conclusions: We have previously demonstrated that ox-LDL produces intracellular reactive oxygen species (ROS) via LOX-1 in articular chondrocytes. And it has been suggested that oxidative stress affects articular chondrocytes, resulting in cell senescence. Further, ROS has been demonstrated to induce chondrocyte hypertrophy in endochondral ossification. In this study, we indicated that ox-LDL up-regulated expression of type X collagen in cultured BACs, which suggests that ox-LDL may make chondrocytes have the hypertrophic chondrocyte-like phenotype by oxidative stress. Furthermore, the results suggest that the HIF family may be involved in this phenomenon. Ox-LDL may play some roles in phenotypic changes of chondrocytes in OA through increase in oxidative stress.

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REGULATION OF CHONDROGENIC DIFFERENTIATION BY NFκβ IN MESENCHYMAL PROGENITOR CELLS

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Purpose: Haematoma formation and injury induced inflammatory responses are essential for bone fracture healing via endochondral ossification. Inflammatory mediators are known to regulate osteoblastogenesis, however it remains unknown whether and how inflammatory responses regulate other stages of the endochondral ossification pathway. We hypothesize that nuclear factor

kappa beta (NFκβ) activation regulates chondrogenic differentiation. This study aims to elucidate the role of NFκβ and downstream inflammatory mediators during the early phase of chondrogenesis.
Methods: The chondroprogenitor ATDC5 was differentiated in presence of NFκβ activating agents LPS and TNFα or with the NFκβ inhibitors TLCK, sulfasalazine or parthenolide. NFκβ activation was determined by analyzing nuclear translocation and expression of NFκβ targets by RT-qPCR and westernblot. Prostaglandin E_2 (PGE_2) production in medium was measured using a specific ELISA. Cartilage specific markers were analyzed by RT-qPCR and western blot. Similar experiments were performed with human bone marrow stem cells (hBMSCs). hBMSCs were obtained from iliac crest bone marrow aspirate from 5 young, genetically healthy, individuals.
Results: ATDC5 cells were triggered for chondrogenesis and during the very early onset of differentiation upregulation of inflammatory mediators COX-2 (and PGE_2), iNOS (and NO production), IL-6 and TNFα was detected (1-8 hours). This inflammatory-response peak was preceded by NFκβ nuclear translocation at 30-60 minutes in differentiation, indicating that expression of these inflammatory mediators might be regulated by NFκβ activation.
To investigate the role of NFκβ activation during early chondrogenesis, NFκβ activation was stimulated by LPS or TNFα. Both lead to upregulation of inflammatory mediators and was accompanied by upregulation of Sox9 at early time points and RunX2, Collagen II and Collagen X late in differentiation. In contrast, inhibition of nuclear translocation of NFκβ by TLCK, sulfasalazine or parthenolide prevented the upregulation of inflammatory mediators at early time points in chondrogenesis. Importantly, inhibition of NFκβ activation also lead to a concentration dependent inhibition of chondrogenic differentiation, strongly suggesting that NFκβ activation is an essential step during early chondrogenesis.
To verify these results, hBMSCs were used as an independent system for chondrogenic differentiation. In concert with our findings in ATDC5 cells, early chondrogenesis of hBMSCs is also accompanied with a similar upregulation of inflammatory mediators like COX-2 and chondrogenic transcription factor Sox9.
Conclusions: During the very early onset of chondrogenesis NFκβ translocates to the nucleus and seems to regulate the expression of inflammatory mediators and the essential chondrogenic transcription factor Sox9. Stimulation of NFκβ lead to an upregulation of inflammatory mediators and enhanced chondrogenic differentiation, whereas inhibition of NFκβ nuclear translocation inhibited chondrogenesis. Our results point to an essential role for NFκβ and inflammatory mediators during the first steps of chondrogenesis, which might regulate Sox9 and subsequent expression of cartilage molecules.
Also, these data show that the effect of NFκβ activation on cartilage might depend on the status of cartilage development and is not solely associated with cartilage degeneration. Importantly, our results might pave the way for alternative strategies in the optimization of osteochondrogenic progenitor cell based cartilage repair techniques.

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BONE AND CARTILAGE DEGRADATION ARE COUPLED UNDER SOME PATHOLOGICAL AND PHYSIOLOGICAL PROCESSES

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Purpose: The pathogenesis of osteoarthritis involves both changes in bone and cartilage. These processes might be coupled under some instances. The aim of the present investigation was to investigate bone and cartilage degradation correlations in osteoarthritis patients as a function of sex, BMI, Kellgren-Lawrence score and diurnal variation.
Methods: The study was a phase-I, 2-week, placebo-controlled, double-blind, double-dummy, randomized, gender-stratified study including 73 subjects (37 postmenopausal women and 36 men), aged 57-75 years. Patients had to have painful OA of at least one knee with a Kellgren-Lawrence index score of I-III. Subjects were allocated to one of three treatment arms: 0.6 mg, 0.8 mg or placebo. Each treatment was given twice-daily for 14 days. For evaluation of pharmacodynamics, blood samples were collected immediately prior to dosing and at ½, 1, 2, 4, 6, and 8 hrs after morning dose and, for the pre-dinner dose, immediately prior to dosing, and at the intervals of ½, 1, 2, and 4 hrs post-dose. Urine samples were collected pre-dose, 2, 4, 6, 8, 13, and 15 hours after morning dose. Study parameters were changes in the bone resorption marker of CTX-I (serum C-terminal telopeptide of collagen type I) and the cartilage degradation marker, CTX-II (urine C-terminal telopeptide of collagen type II).
Results: A significant difference ($p=0.04$) in CTX-I between men and women, and a borderline significant difference between men and women in CTX-II ($p=0.06$) was found. At baseline, a correlation between CTX-I and KL-score were not found. In contrast CTX-II levels were significantly correlated to increasing KL-score in both men and women ($P=0.007$). BMI and CTX-I was negatively correlated $r = -0.40$ ($p=0.002$), whereas CTX-II showed a weak and non-significant positive correlation with BMI, $r = 0.25$ ($p=0.12$). No correlation between CTX-I and CTX-II was found before morning dosing at day 1 and 14. 4 hours after dosing, a significant correlation ($r=0.71$, $p<0.001$) between changes in CTX-I and CTX-II was seen in the sCT group on both day 1 and 14, whereas in the placebo group a weakly significant correlation was found on day 1 ($r=0.49$, $p=0.02$), but not day 14. CTX-I displayed marked diurnal variation in which a more than 50% decrease was seen as a response to intake of lunch in the placebo group. CTX-II displayed lower levels of diurnal variation, indicating that CTX-II levels also are postprandial regulated.
Conclusions: Bone and cartilage degradation may be coupled processes. Understanding of the physiological and pathological situations in which coupling and uncoupling are present may aid in the identification of treatment opportunities for osteoarthritis. We speculate that identification of physiological processes in which both bone and cartilage turnover are attenuated may aid in the identification of new treatment opportunities for osteoarthritis.

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HUMAN ARTICULAR CHONDROCYTES EXPRESS 15-LIPOXYGENASE-1 AND -2: POTENTIAL ROLE IN OSTEOARTHRITIS

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Purpose: To evaluate the expression of 15-lipoxygenase (LOX)-1 and -2 in articular chondrocytes and to investigate the effects of their metabolites, 13-HODE and 15-HETE, on IL-1-induced MMP-1 and -13 expression.
Methods: The expression of 15-LOX-1 and -2 was analyzed by reverse transcriptase-polymerase chain reaction (RT-PCR) and Western blotting in chondrocytes, and by immunohistochemistry in cartilage. Chondrocytes were stimulated with IL-1 in the absence or presence of 13-HODE and 15-HETE and the level of MMP-1 and -13 protein and mRNA expression were evaluated by immunoassay and real-time RT-PCR, respectively. The role of